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PCT/GB 2005 / 0 0 0 9 1 5

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Dated 11 April 2005

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

THE PATENT OFFICE
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- 9 MAR 2004
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The Patent Office

Cardiff Road
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1. Your reference

RJW/LP6212088

2. Patent application number
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0405319.5

- 9 MAR 2004

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

SPIROGEN LIMITED
79 George Street
Ryde
ISLE OF WIGHT
PO33 2JF

Patents ADP number *(if you know it)*

8051872001

If the applicant is a corporate body, give the country/state of its incorporation

England

4. Title of the invention

PYRROLOBENZODIAZEPINES

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

MEWBURN ELLIS
York House
23 Kingsway
London WC2B 6HP

Patents ADP number *(if you know it)*

109006

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months.

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute (see note f)

Number of earlier UK application	Date of filing *(day / month / year)*

8. Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?

YES

Answer YES if:

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.

Otherwise answer NO (See note d)

Patents Form 1/77

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form	
Description	39
Claim(s)	
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	
Request for a preliminary examination and search (Patents Form 9/77)	
Request for a substantive examination (Patents Form 10/77)	
Any other documents (please specify)	

11. I/We request the grant of a patent on the basis of this application.

Signature(s) Mark Ellis Date 9 March 2004

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

ROBERT WATSON
020 7240 4405

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Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

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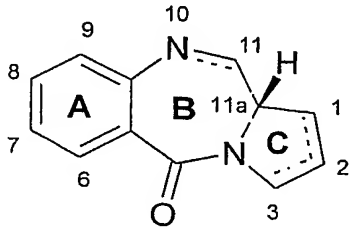
PYRROLOBENZODIAZEPINES

The present invention relates to a specific pyrrolobenzodiazepine (PBD) dimer with C2-exo unsaturation.

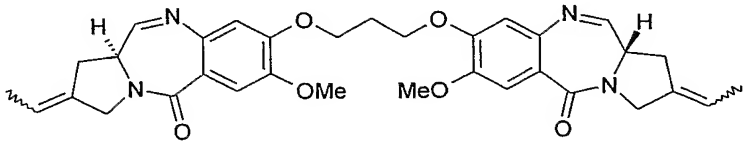
Background to the invention

Some pyrrolobenzodiazepines (PBDs) have the ability to recognise and bond to specific sequences of DNA; the preferred sequence is PuGPu. The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber, *et al., J. Am. Chem. Soc.*, **87**, 5793-5795 (1965); Leimgruber, *et al., J. Am. Chem. Soc.*, **87**, 5791-5793 (1965)). Since then, a number of naturally occurring PBDs have been reported, and over 10 synthetic routes have been developed to a variety of analogues (Thurston, *et al., Chem. Rev.* **1994**, 433-465 (1994)). Family members include abbeymycin (Hochlowski, *et al., J. Antibiotics*, **40**, 145-148 (1987)), chicamycin (Konishi, *et al., J. Antibiotics*, **37**, 200-206 (1984)), DC-81 (Japanese Patent 58-180 487; Thurston, *et al., Chem. Brit.*, **26**, 767-772 (1990); Bose, *et al., Tetrahedron*, **48**, 751-758 (1992)), mazethramycin (Kuminoto, *et al., J. Antibiotics*, **33**, 665-667 (1980)), neothramycins A and B (Takeuchi, *et al., J. Antibiotics*, **29**, 93-96 (1976)), porothramycin (Tsunakawa, *et al., J. Antibiotics*, **41**, 1366-1373 (1988)), prothracarcin (Shimizu, *et al, J. Antibiotics*, **29**, 2492-2503 (1982); Langley and Thurston, *J. Org. Chem.*, **52**, 91-97 (1987)), sibanomicin (DC-102)(Hara, *et al., J. Antibiotics*, **41**, 702-704 (1988); Itoh, *et al., J. Antibiotics*, **41**, 1281-1284 (1988)), sibiromycin (Leber, *et al., J. Am. Chem. Soc.*, **110**, 2992-2993 (1988)) and tomamycin (Arima, *et al., J. Antibiotics*, **25**, 437-444 (1972)). PBDs are of the general structure:



They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-CH(OMe)) at the N10-C11 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (*S*)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In *Antibiotics III*. Springer-Verlag, New York, pp. 3-11 (1975); Hurley and Needham-VanDevanter, *Acc. Chem. Res.*, **19**, 230-237 (1986)). Their ability to form an adduct in the minor groove, enables them to interfere with DNA processing, hence their use as antitumour agents.

In WO 93/18045, some of the present inventors disclosed the following compound (Example 6):

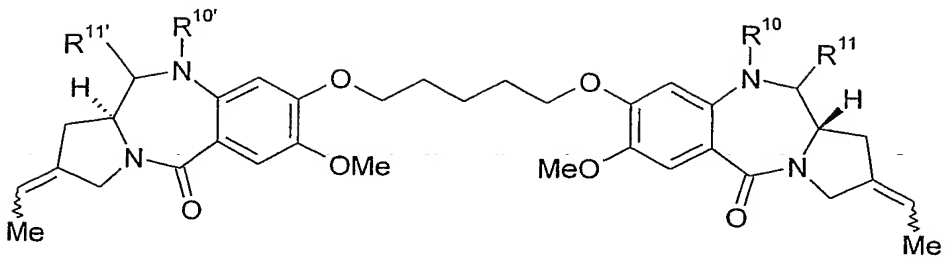


The final compound produced was a mixture of the E-, E- form, the Z-, Z- form and the E-, Z- forms as a result of the synthesis method used. Extrapolating from the last compound for which the amount of different geometric isomers was measured, the final compound would likely have the following proportions of geometric isomers:

Geometric isomers at C2/C2′	Amount (%)
E-, E-	42
E-, Z-	46
Z-, Z-	12

Disclosure of the invention

In a first aspect, the invention comprises a compound of formula **I**:



(I)

and salts and solvates thereof, wherein:

R^{10} is a nitrogen protecting group and R^{11} is either OH or O-R^{12}, wherein R^{12} is an oxygen protecting group, or R^{10} and R^{11} together form a double bond between N10 and C11;

and $R^{10'}$ and $R^{11'}$ are selected from the same options as R^{10} and R^{11} respectively.

It is preferred that $R^{10'}$ and $R^{11'}$ are the same as R^{10} and R^{11} respectively.

In a second aspect, the invention comprises the synthesis of a compound of formula **I**.

In a third aspect, the invention comprises a compound of formula **I** and pharmaceutically acceptable salts and solvates thereof, for use in a method of therapy.

In a fourth aspect, the invention comprises a pharmaceutical composition comprising a compound of formula **I** and pharmaceutically acceptable salts and solvates thereof, and a pharmaceutically acceptable excipient.

In a fifth aspect, the invention comprises the use of a compound of formula **I** and pharmaceutically acceptable salts and solvates thereof, in the manufacture of a medicament for the treatment of a gene-based disease.

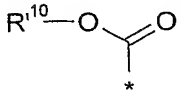
In a sixth aspect, the invention comprises a method for the treatment of a gene-based disease, comprising administering to a subject suffering from a gene-based disease a therapeutically-

effective amount of a compound of formula **I** or pharmaceutically acceptable salts and solvates thereof.

Definitions

Nitrogen protecting groups

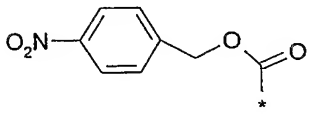
Nitrogen protecting groups are well known in the art. Preferred nitrogen protecting groups are carbamate protecting groups that have the general formula:



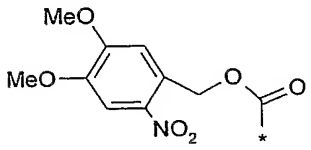
A large number of possible carbamate nitrogen protecting groups are listed on pages 503 to 549 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference.

Particularly preferred protecting groups include Alloc, Troc, Teoc, BOC, Doc, Hoc, TcBOC, Fmoc, 1-Adoc and 2-Adoc.

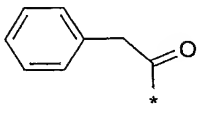
Also suitable for use in the present invention are nitrogen protecting groups which can be removed *in vivo* (e.g. enzymatically, using light) as described in WO 00/12507, which is incorporated herein by reference. Examples of these protecting groups include:



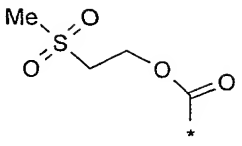
, which is nitroreductase labile (e.g. using ADEPT/GDEPT);



and



, which are photolabile; and



which is glutathione labile (e.g. using NPEPT).

Oxygen protecting groups

Oxygen protecting groups are well known in the art. A large number of suitable groups are described on pages 23 to 200 of Greene, T.W. and Wuts, G.M., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons, Inc., 1999, which is incorporated herein by reference.

Classes of particular interest include silyl ethers, methyl ethers, alkyl ethers, benzyl ethers, esters, benzoates, carbonates, and sulfonates.

Substituents

The phrase "optionally substituted" as used herein, pertains to a parent group which may be unsubstituted or which may be substituted.

Unless otherwise specified, the term "substituted" as used herein, pertains to a parent group which bears one or more substitutents. The term "substituent" is used herein in the conventional sense and refers to a chemical moiety which is covalently attached to, or if appropriate, fused to, a parent group. A wide variety of substituents are well known, and methods for their formation and introduction into a variety of parent groups are also well known.

Examples of substituents are described in more detail below.

C_{1-7} alkyl: The term "C_{1-7} alkyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 7 carbon atoms, which may be aliphatic or alicyclic, and which may be saturated or unsaturated (e.g. partially unsaturated, fully

unsaturated). Thus, the term "alkyl" includes the sub-classes alkenyl, alkynyl, cycloalkyl, etc., discussed below.

Examples of saturated alkyl groups include, but are not limited to, methyl (C_1), ethyl (C_2), propyl (C_3), butyl (C_4), pentyl (C_5), hexyl (C_6) and heptyl (C_7).

Examples of saturated linear alkyl groups include, but are not limited to, methyl (C_1), ethyl (C_2), n-propyl (C_3), n-butyl (C_4), n-pentyl (amyl) (C_5), n-hexyl (C_6) and n-heptyl (C_7).

Examples of saturated branched alkyl groups include iso-propyl (C_3), iso-butyl (C_4), sec-butyl (C_4), tert-butyl (C_4), iso-pentyl (C_5), and neo-pentyl (C_5).

C_{2-7} Alkenyl: The term "C_{2-7} alkenyl" as used herein, pertains to an alkyl group having one or more carbon-carbon double bonds.

Examples of unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, $-CH=CH_2$), 1-propenyl ($-CH=CH-CH_3$), 2-propenyl (allyl, $-CH-CH=CH_2$), isopropenyl (1-methylvinyl, $-C(CH_3)=CH_2$), butenyl (C_4), pentenyl (C_5), and hexenyl (C_6).

C_{2-7} alkynyl: The term "C_{2-7} alkynyl" as used herein, pertains to an alkyl group having one or more carbon-carbon triple bonds.

Examples of unsaturated alkynyl groups include, but are not limited to, ethynyl (ethinyl, $-C\equiv CH$) and 2-propynyl (propargyl, $-CH_2-C\equiv CH$).

C_{3-7} cycloalkyl: The term "C_{3-7} cycloalkyl" as used herein, pertains to an alkyl group which is also a cyclyl group; that is, a monovalent moiety obtained by removing a hydrogen atom from an alicyclic ring atom of a cyclic hydrocarbon (carbocyclic) compound, which moiety has from 3 to 7 carbon atoms, including from 3 to 7 ring atoms.

Examples of cycloalkyl groups include, but are not limited to, those derived from:

saturated monocyclic hydrocarbon compounds: cyclopropane (C_3), cyclobutane (C_4), cyclopentane (C_5), cyclohexane (C_6), cycloheptane (C_7), methylcyclopropane (C_4), dimethylcyclopropane (C_5), methylcyclobutane (C_5), dimethylcyclobutane (C_6), methylcyclopentane (C_6), dimethylcyclopentane (C_7) and methylcyclohexane (C_7);

unsaturated monocyclic hydrocarbon compounds: cyclopropene (C_3), cyclobutene (C_4), cyclopentene (C_5), cyclohexene (C_6), methylcyclopropene (C_4), dimethylcyclopropene (C_5), methylcyclobutene (C_5), dimethylcyclobutene (C_6), methylcyclopentene (C_6), dimethylcyclopentene (C_7) and methylcyclohexene (C_7); and

saturated polycyclic hydrocarbon compounds: norcarane (C_7), norpinane (C_7), norbornane (C_7).

C_{3-20} heterocyclyl: The term "C_{3-20} heterocyclyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a heterocyclic compound, which moiety has from 3 to 20 ring atoms, of which from 1 to 10 are ring heteroatoms. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms.

In this context, the prefixes (e.g. C_{3-20}, C_{3-7}, C_{5-6}, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term "C_{5-6}heterocyclyl", as used herein, pertains to a heterocyclyl group having 5 or 6 ring atoms.

Examples of monocyclic heterocyclyl groups include, but are not limited to, those derived from:

N_1: aziridine (C_3), azetidine (C_4), pyrrolidine (tetrahydropyrrole) (C_5), pyrroline (e.g., 3-pyrroline, 2,5-dihydropyrrole) (C_5), 2H-pyrrole or 3H-pyrrole (isopyrrole, isoazole) (C_5), piperidine (C_6), dihydropyridine (C_6), tetrahydropyridine (C_6), azepine (C_7);

O_1: oxirane (C_3), oxetane (C_4), oxolane (tetrahydrofuran) (C_5), oxole (dihydrofuran) (C_5), oxane (tetrahydropyran) (C_6), dihydropyran (C_6), pyran (C_6), oxepin (C_7);
S_1: thiirane (C_3), thietane (C_4), thiolane (tetrahydrothiophene) (C_5), thiane (tetrahydrothiopyran) (C_6), thiepane (C_7);
O_2: dioxolane (C_5), dioxane (C_6), and dioxepane (C_7);
O_3: trioxane (C_6);
N_2: imidazolidine (C_5), pyrazolidine (diazolidine) (C_5), imidazoline (C_5), pyrazoline (dihydropyrazole) (C_5), piperazine (C_6);
N_1O_1: tetrahydrooxazole (C_5), dihydrooxazole (C_5), tetrahydroisoxazole (C_5), dihydroisoxazole (C_5), morpholine (C_6), tetrahydrooxazine (C_6), dihydrooxazine (C_6), oxazine (C_6);
N_1S_1: thiazoline (C_5), thiazolidine (C_5), thiomorpholine (C_6);
N_2O_1: oxadiazine (C_6);
O_1S_1: oxathiole (C_5) and oxathiane (thioxane) (C_6); and,
$N_1O_1S_1$: oxathiazine (C_6).

Examples of substituted monocyclic heterocyclyl groups include those derived from saccharides, in cyclic form, for example, furanoses (C_5), such as arabinofuranose, lyxofuranose, ribofuranose, and xylofuranse, and pyranoses (C_6), such as allopyranose, altropyranose, glucopyranose, mannopyranose, gulopyranose, idopyranose, galactopyranose, and talopyranose.

C_{5-20} aryl: The term "C_{5-20} aryl", as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 3 to 20 ring atoms. Preferably, each ring has from 5 to 7 ring atoms.

In this context, the prefixes (e.g. C_{3-20}, C_{5-7}, C_{5-6}, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term "C_{5-6} aryl" as used herein, pertains to an aryl group having 5 or 6 ring atoms.

The ring atoms may be all carbon atoms, as in "carboaryl groups". Examples of carboaryl groups include, but are not limited to, those derived from benzene (i.e. phenyl) (C_6), naphthalene (C_{10}), azulene (C_{10}), anthracene (C_{14}), phenanthrene (C_{14}), naphthacene (C_{18}), and pyrene (C_{16}).

Examples of aryl groups which comprise fused rings, at least one of which is an aromatic ring, include, but are not limited to, groups derived from indane (e.g. 2,3-dihydro-1H-indene) (C_9), indene (C_9), isoindene (C_9), tetraline (1,2,3,4-tetrahydronaphthalene (C_{10}), acenaphthene (C_{12}), fluorene (C_{13}), phenalene (C_{13}), acephenanthrene (C_{15}), and aceanthrene (C_{16}).

Alternatively, the ring atoms may include one or more heteroatoms, as in "heteroaryl groups". Examples of monocyclic heteroaryl groups include, but are not limited to, those derived from:

N_1: pyrrole (azole) (C_5), pyridine (azine) (C_6);

O_1: furan (oxole) (C_5);

S_1: thiophene (thiole) (C_5);

N_1O_1: oxazole (C_5), isoxazole (C_5), isoxazine (C_6);

N_2O_1: oxadiazole (furazan) (C_5);

N_3O_1: oxatriazole (C_5);

N_1S_1: thiazole (C_5), isothiazole (C_5);

N_2: imidazole (1,3-diazole) (C_5), pyrazole (1,2-diazole) (C_5), pyridazine (1,2-diazine) (C_6), pyrimidine (1,3-diazine) (C_6) (e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) (C_6);

N_3: triazole (C_5), triazine (C_6); and,

N_4: tetrazole (C_5).

Examples of heteroaryl which comprise fused rings, include, but are not limited to:

C_9 (with 2 fused rings) derived from benzofuran (O_1), isobenzofuran (O_1), indole (N_1), isoindole (N_1), indolizine (N_1), indoline (N_1), isoindoline (N_1), purine (N_4) (e.g., adenine, guanine), benzimidazole (N_2), indazole (N_2), benzoxazole (N_1O_1),

benzisoxazole (N_1O_1), benzodioxole (O_2), benzofurazan (N_2O_1), benzotriazole (N_3), benzothiofuran (S_1), benzothiazole (N_1S_1), benzothiadiazole (N_2S);

C_{10} (with 2 fused rings) derived from chromene (O_1), isochromene (O_1), chroman (O_1), isochroman (O_1), benzodioxan (O_2), quinoline (N_1), isoquinoline (N_1), quinolizine (N_1), benzoxazine (N_1O_1), benzodiazine (N_2), pyridopyridine (N_2), quinoxaline (N_2), quinazoline (N_2), cinnoline (N_2), phthalazine (N_2), naphthyridine (N_2), pteridine (N_4);

C_{11} (with 2 fused rings) derived from benzodiazepine (N_2);

C_{13} (with 3 fused rings) derived from carbazole (N_1), dibenzofuran (O_1), dibenzothiophene (S_1), carboline (N_2), perimidine (N_2), pyridoindole (N_2); and,

C_{14} (with 3 fused rings) derived from acridine (N_1), xanthene (O_1), thioxanthene (S_1), oxanthrene (O_2), phenoxathiin (O_1S_1), phenazine (N_2), phenoxazine (N_1O_1), phenothiazine (N_1S_1), thianthrene (S_2), phenanthridine (N_1), phenanthroline (N_2), phenazine (N_2).

The above groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkoxy group, discussed below), a C_{3-20} heterocyclyl group (also referred to as a C_{3-20} heterocyclyloxy group), or a C_{5-20} aryl group (also referred to as a C_{5-20} aryloxy group), preferably a C_{1-7}alkyl group.

Alkoxy: -OR, wherein R is an alkyl group, for example, a C_{1-7} alkyl group. Examples of C_{1-7} alkoxy groups include, but are not limited to, -OMe (methoxy), -OEt (ethoxy), -O(nPr) (n-propoxy), -

O(iPr) (isopropoxy), -O(nBu) (n-butoxy), -O(sBu) (sec-butoxy), -O(iBu) (isobutoxy), and -O(tBu) (tert-butoxy).

Acetal: $-CH(OR^1)(OR^2)$, wherein R^1 and R^2 are independently acetal substituents, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group, or, in the case of a "cyclic" acetal group, R^1 and R^2, taken together with the two oxygen atoms to which they are attached, and the carbon atoms to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of acetal groups include, but are not limited to, $-CH(OMe)_2$, $-CH(OEt)_2$, and -CH(OMe)(OEt).

Hemiacetal: $-CH(OH)(OR^1)$, wherein R^1 is a hemiacetal substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of hemiacetal groups include, but are not limited to, -CH(OH)(OMe) and -CH(OH)(OEt).

Ketal: $-CR(OR^1)(OR^2)$, where R^1 and R^2 are as defined for acetals, and R is a ketal substituent other than hydrogen, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples ketal groups include, but are not limited to, $-C(Me)(OMe)_2$, $-C(Me)(OEt)_2$, -C(Me)(OMe)(OEt), $-C(Et)(OMe)_2$, $-C(Et)(OEt)_2$, and -C(Et)(OMe)(OEt).

Hemiketal: $-CR(OH)(OR^1)$, where R^1 is as defined for hemiacetals, and R is a hemiketal substituent other than hydrogen, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of hemiacetal groups include, but are not limited to, -C(Me)(OH)(OMe), -C(Et)(OH)(OMe), -C(Me)(OH)(OEt), and -C(Et)(OH)(OEt).

Oxo (keto, -one): =O.

Thione (thioketone): =S.

Imino (imine): =NR, wherein R is an imino substituent, for example, hydrogen, C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, =NH, =NMe, =NEt, and =NPh.

Formyl (carbaldehyde, carboxaldehyde): -C(=O)H.

Acyl (keto): -C(=O)R, wherein R is an acyl substituent, for example, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylacyl or C_{1-7} alkanoyl), a C_{3-20} heterocyclyl group (also referred to as C_{3-20} heterocyclylacyl), or a C_{5-20} aryl group (also referred to as C_{5-20} arylacyl), preferably a C_{1-7} alkyl group. Examples of acyl groups include, but are not limited to, -C(=O)CH_3 (acetyl), -C(=O)CH_2CH_3 (propionyl), -C(=O)$C(CH_3)_3$ (t-butyryl), and -C(=O)Ph (benzoyl, phenone).

Carboxy (carboxylic acid): -C(=O)OH.

Thiocarboxy (thiocarboxylic acid): -C(=S)SH.

Thiolocarboxy (thiolocarboxylic acid): -C(=O)SH.

Thionocarboxy (thionocarboxylic acid): -C(=S)OH.

Imidic acid: -C(=NH)OH.

Hydroxamic acid: -C(=NOH)OH.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=O)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, -C(=O)OCH_3, -C(=O)OCH_2CH_3, -C(=O)$OC(CH_3)_3$, and -C(=O)OPh.

Acyloxy (reverse ester): -OC(=O)R, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of acyloxy groups include, but are not limited to, $-OC(=O)CH_3$ (acetoxy), $-OC(=O)CH_2CH_3$, $-OC(=O)C(CH_3)_3$, -OC(=O)Ph, and $-OC(=O)CH_2Ph$.

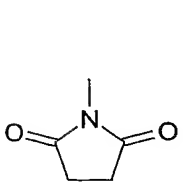
Oxycarboyloxy: -OC(=O)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, $-OC(=O)OCH_3$, $-OC(=O)OCH_2CH_3$, $-OC(=O)OC(CH_3)_3$, and -OC(=O)OPh.

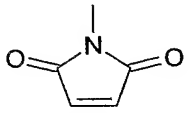
Amino: $-NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, or, in the case of a "cyclic" amino group, R^1 and R^2, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Amino groups may be primary ($-NH_2$), secondary ($-NHR^1$), or tertiary ($-NHR^1R^2$), and in cationic form, may be quaternary ($-^+NR^1R^2R^3$). Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHC(CH_3)_2$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, and -NHPh. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.

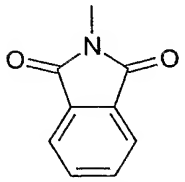
Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which R^1 and R^2, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

Thioamido (thiocarbamyl): $-C(=S)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=S)NH_2$, $-C(=S)NHCH_3$, $-C(=S)N(CH_3)_2$, and $-C(=S)NHCH_2CH_3$.

Acylamido (acylamino): $-NR^1C(=O)R^2$, wherein R^1 is an amide substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group, and R^2 is an acyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20}aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of acylamide groups include, but are not limited to, $-NHC(=O)CH_3$, $-NHC(=O)CH_2CH_3$, and $-NHC(=O)Ph$. R^1 and R^2 may together form a cyclic structure, as in, for example, succinimidyl, maleimidyl, and phthalimidyl:


succinimidyl


maleimidyl

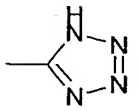

phthalimidyl

Aminocarbonyloxy: $-OC(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of aminocarbonyloxy groups include, but are not limited to, $-OC(=O)NH_2$, $-OC(=O)NHMe$, $-OC(=O)NMe_2$, and $-OC(=O)NEt_2$.

Ureido: $-N(R^1)CONR^2R^3$ wherein R^2 and R^3 are independently amino substituents, as defined for amino groups, and R^1 is a ureido substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of ureido groups include, but are not limited to, $-NHCONH_2$, $-NHCONHMe$, $-NHCONHEt$, $-NHCONMe_2$, $-NHCONEt_2$, $-NMeCONH_2$, $-NMeCONHMe$, $-NMeCONHEt$, $-NMeCONMe_2$, and $-NMeCONEt_2$.

Guanidino: $-NH-C(=NH)NH_2$.

Tetrazolyl: a five membered aromatic ring having four nitrogen atoms and one carbon atom,



Imino: =NR, wherein R is an imino substituent, for example, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7}alkyl group. Examples of imino groups include, but are not limited to, =NH, =NMe, and =NEt.

Amidine (amidino): $-C(=NR)NR_2$, wherein each R is an amidine substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group. Examples of amidine groups include, but are not limited to, $-C(=NH)NH_2$, $-C(=NH)NMe_2$, and $-C(=NMe)NMe_2$.

Nitro: $-NO_2$.

Nitroso: -NO.

Azido: $-N_3$.

Cyano (nitrile, carbonitrile): -CN.

Isocyano: -NC.

Cyanato: -OCN.

Isocyanato: -NCO.

Thiocyano (thiocyanato): -SCN.

Isothiocyano (isothiocyanato): -NCS.

Sulfhydryl (thiol, mercapto): -SH.

Thioether (sulfide): -SR, wherein R is a thioether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7}alkylthio group), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of C_{1-7} alkylthio groups include, but are not limited to, $-SCH_3$ and $-SCH_2CH_3$.

Disulfide: -SS-R, wherein R is a disulfide substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group (also referred to herein as C_{1-7} alkyl disulfide). Examples of C_{1-7} alkyl disulfide groups include, but are not limited to, $-SSCH_3$ and $-SSCH_2CH_3$.

Sulfine (sulfinyl, sulfoxide): $-S(=O)R$, wherein R is a sulfine substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfine groups include, but are not limited to, $-S(=O)CH_3$ and $-S(=O)CH_2CH_3$.

Sulfone (sulfonyl): $-S(=O)_2R$, wherein R is a sulfone substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group, including, for example, a fluorinated or perfluorinated C_{1-7} alkyl group. Examples of sulfone groups include, but are not limited to, $-S(=O)_2CH_3$ (methanesulfonyl, mesyl), $-S(=O)_2CF_3$ (triflyl), $-S(=O)_2CH_2CH_3$ (esyl), $-S(=O)_2C_4F_9$ (nonaflyl), $-S(=O)_2CH_2CF_3$ (tresyl), $-S(=O)_2CH_2CH_2NH_2$ (tauryl), $-S(=O)_2Ph$ (phenylsulfonyl, besyl), 4-methylphenylsulfonyl (tosyl), 4-chlorophenylsulfonyl (closyl), 4-bromophenylsulfonyl (brosyl), 4-nitrophenyl (nosyl), 2-naphthalenesulfonate (napsyl), and 5-dimethylamino-naphthalen-1-ylsulfonate (dansyl).

Sulfinic acid (sulfino): $-S(=O)OH$, $-SO_2H$.

Sulfonic acid (sulfo): $-S(=O)_2OH$, $-SO_3H$.

Sulfinate (sulfinic acid ester): -S(=O)OR; wherein R is a sulfinate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinate groups include, but are not limited to, $-S(=O)OCH_3$ (methoxysulfinyl; methyl sulfinate) and $-S(=O)OCH_2CH_3$ (ethoxysulfinyl; ethyl sulfinate).

Sulfonate (sulfonic acid ester): $-S(=O)_2OR$, wherein R is a sulfonate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonate groups include, but are not limited to, $-S(=O)_2OCH_3$ (methoxysulfonyl; methyl sulfonate) and $-S(=O)_2OCH_2CH_3$ (ethoxysulfonyl; ethyl sulfonate).

Sulfinyloxy: $-OS(=O)R$, wherein R is a sulfinyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinyloxy groups include, but are not limited to, $-OS(=O)CH_3$ and $-OS(=O)CH_2CH_3$.

Sulfonyloxy: $-OS(=O)_2R$, wherein R is a sulfonyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonyloxy groups include, but are not limited to, $-OS(=O)_2CH_3$ (mesylate) and $-OS(=O)_2CH_2CH_3$ (esylate).

Sulfate: $-OS(=O)_2OR$; wherein R is a sulfate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfate groups include, but are not limited to, $-OS(=O)_2OCH_3$ and $-SO(=O)_2OCH_2CH_3$.

Sulfamyl (sulfamoyl; sulfinic acid amide; sulfinamide): $-S(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to, $-S(=O)NH_2$, $-S(=O)NH(CH_3)$, $-S(=O)N(CH_3)_2$, $-S(=O)NH(CH_2CH_3)$, $-S(=O)N(CH_2CH_3)_2$, and $-S(=O)NHPh$.

Sulfonamido (sulfinamoyl; sulfonic acid amide; sulfonamide): $-S(=O)_2NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of sulfonamido groups include, but are not limited to, $-S(=O)_2NH_2$, $-S(=O)_2NH(CH_3)$, $-S(=O)_2N(CH_3)_2$, $-S(=O)_2NH(CH_2CH_3)$, $-S(=O)_2N(CH_2CH_3)_2$, and $-S(=O)_2NHPh$.

Sulfamino: $-NR^1S(=O)_2OH$, wherein R^1 is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to, $-NHS(=O)_2OH$ and $-N(CH_3)S(=O)_2OH$.

Sulfonamino: $-NR^1S(=O)_2R$, wherein R^1 is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonamino groups include, but are not limited to, $-NHS(=O)_2CH_3$ and $-N(CH_3)S(=O)_2C_6H_5$.

Sulfinamino: $-NR^1S(=O)R$, wherein R^1 is an amino substituent, as defined for amino groups, and R is a sulfinamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinamino groups include, but are not limited to, $-NHS(=O)CH_3$ and $-N(CH_3)S(=O)C_6H_5$.

Phosphino (phosphine): $-PR_2$, wherein R is a phosphino substituent, for example, -H, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphino groups include, but are not limited to, $-PH_2$, $-P(CH_3)_2$, $-P(CH_2CH_3)_2$, $-P(t\text{-}Bu)_2$, and $-P(Ph)_2$.

Phospho: $-P(=O)_2$.

Phosphinyl (phosphine oxide): $-P(=O)R_2$, wherein R is a phosphinyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl

group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group or a C_{5-20} aryl group. Examples of phosphinyl groups include, but are not limited to, $-P(=O)(CH_3)_2$, $-P(=O)(CH_2CH_3)_2$, $-P(=O)(t-Bu)_2$, and $-P(=O)(Ph)_2$.

Phosphonic acid (phosphono): $-P(=O)(OH)_2$.

Phosphonate (phosphono ester): $-P(=O)(OR)_2$, where R is a phosphonate substituent, for example, -H, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphonate groups include, but are not limited to, $-P(=O)(OCH_3)_2$, $-P(=O)(OCH_2CH_3)_2$, $-P(=O)(O-t-Bu)_2$, and $-P(=O)(OPh)_2$.

Phosphoric acid (phosphonooxy): $-OP(=O)(OH)_2$.

Phosphate (phosphonooxy ester): $-OP(=O)(OR)_2$, where R is a phosphate substituent, for example, -H, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphate groups include, but are not limited to, $-OP(=O)(OCH_3)_2$, $-OP(=O)(OCH_2CH_3)_2$, $-OP(=O)(O-t-Bu)_2$, and $-OP(=O)(OPh)_2$.

Phosphorous acid: $-OP(OH)_2$.

Phosphite: $-OP(OR)_2$, where R is a phosphite substituent, for example, -H, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphite groups include, but are not limited to, $-OP(OCH_3)_2$, $-OP(OCH_2CH_3)_2$, $-OP(O-t-Bu)_2$, and $-OP(OPh)_2$.

Phosphoramidite: $-OP(OR^1)-NR^2_2$, where R^1 and R^2 are phosphoramidite substituents, for example, -H, a (optionally substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphoramidite groups include, but are not limited to,

$-OP(OCH_2CH_3)-N(CH_3)_2$, $-OP(OCH_2CH_3)-N(i-Pr)_2$, and $-OP(OCH_2CH_2CN)-N(i-Pr)_2$.

Phosphoramidate: $-OP(=O)(OR^1)-NR^2_2$, where R^1 and R^2 are phosphoramidate substituents, for example, -H, a (optionally substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphoramidate groups include, but are not limited to, $-OP(=O)(OCH_2CH_3)-N(CH_3)_2$, $-OP(=O)(OCH_2CH_3)-N(i-Pr)_2$, and $-OP(=O)(OCH_2CH_2CN)-N(i-Pr)_2$.

Gene-based diseases

Gene-based diseases include, and are preferably, proliferative diseases, and also include Alzheimer's disease and bacterial, parasitic and viral infections. Any condition which may be treated by the regulation of gene expression may be treated the compounds of the present invention.

Proliferative Diseases

One of ordinary skill in the art is readily able to determine whether or not a candidate compound treats a proliferative condition for any particular cell type. For example, assays which may conveniently be used to assess the activity offered by a particular compound are described in the examples below.

The term "proliferative disease" pertains to an unwanted or uncontrolled cellular proliferation of excessive or abnormal cells which is undesired, such as, neoplastic or hyperplastic growth, whether *in vitro* or *in vivo*.

Examples of proliferative conditions include, but are not limited to, benign, pre-malignant, and malignant cellular proliferation, including but not limited to, neoplasms and tumours (e.g. histocytoma, glioma, astrocyoma, osteoma), cancers (e.g. lung cancer, small cell lung cancer, gastrointestinal cancer, bowel cancer, colon cancer, breast carinoma, ovarian carcinoma,

prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreas cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, melanoma), leukemias, psoriasis, bone diseases, fibroproliferative disorders (e.g. of connective tissues), and atherosclerosis.

Any type of cell may be treated, including but not limited to, lung, gastrointestinal (including, e.g. bowel, colon), breast (mammary), ovarian, prostate, liver (hepatic), kidney (renal), bladder, pancreas, brain, and skin.

Methods of Treatment

As described above, the present invention provide the use of a compound of formula **I** in a method of therapy. Preferably the compounds of formula **I** for use in therapy comprise two N10-C11 imine bonds, or the N10s are protected by nitrogen protecting groups (R^{10}, $R^{10'}$) which can be removed in vivo and the C11 substituents (R^{11}, $R^{11'}$) are OH. Also provided is a method of treatment, comprising administering to a subject in need of treatment a therapeutically-effective amount of a compound of formula **I**, preferably in the form of a pharmaceutical composition, which is the third aspect of the present invention. The term "therapeutically effective amount" is an amount sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage, is within the responsibility of general practitioners and other medical doctors.

A compound may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Examples of treatments and therapies include, but are not limited to, chemotherapy (the administration of active agents, including, e.g. drugs; surgery; and radiation therapy. If the compound of formula **I** bears a

carbamate-based nitrogen protecting group which may be removed *in vivo*, then the methods of treatment described in WO 00/12507 (ADEPT, GDEPT and PDT) may be used.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the active ingredient, i.e. a compound of formula **I**, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous, or intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such a gelatin.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

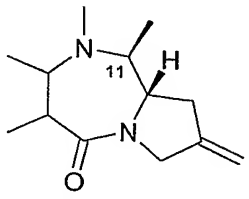
Includes Other Forms

Unless otherwise specified, included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form ($-COO^-$), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form ($-N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form ($-O^-$), a salt or solvate thereof, as well as conventional protected forms.

Isomers, Salts and Solvates

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, atropic, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

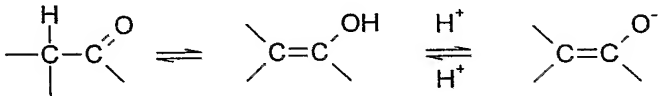
Preferably compounds of the present invention have the following stereochemistry at the C11 position:



Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers", as used herein, are structural (or constitutional) isomers (i.e. isomers which

differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, $-OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $-CH_2OH$. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g. C_{1-7} alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.



Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^{1}H, ^{2}H (D), and ^{3}H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g. fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, *et al.*, *J. Pharm. Sci.*, **66**, 1-19 (1977).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g. -COOH may be $-COO^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+, alkaline earth cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{+3}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e. NH_4^+) and substituted ammonium ions (e.g. NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

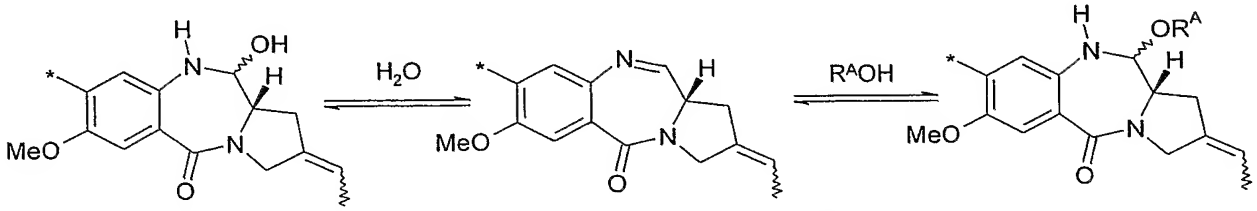
If the compound is cationic, or has a functional group which may be cationic (e.g. $-NH_2$ may be $-NH_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic,

ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pamoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, and valeric. Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

Solvates of particular relevance to the present invention are those where the solvent adds across the imine bond of the PBD, which is illustrated below where the solvent is water or an alcohol (R^AOH, where R^A is an ether substituent as described above):



wherein, * indicates the dimer bridge ($-O-(CH_2)_5-O-$) to the corresponding PBD unit.

These forms can be called the carbinolamine and carbinolamine ether forms of the PBD. The balance of these equilibria depend on the conditions in which the compounds are found, as well as the nature of the moiety itself.

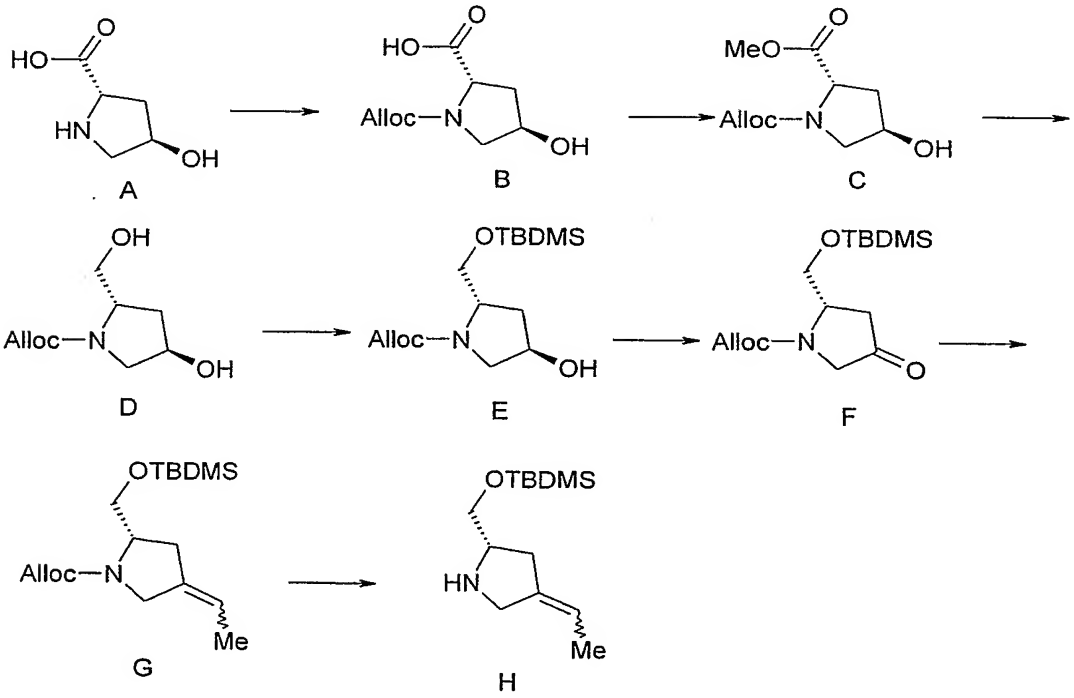
In general any nuceleophilic solvent is capable of forming such solvates as illustrated above for hydoxylic solvents. Other nuceleophilic solvents include thiols and amines.

These solvates may be isolated in solid form, for example, by lyophilisation.

General synthetic routes

The compounds of formula **I** may be made by two alternative routes which are similar to those described in WO 00/12508. An important step is the formation of the C2-exo double bond. This may proceed by the methods described in schemes 8 and 9 of WO 00/12508.

One method involves the synthesis of the compound to provide the C-ring before coupling to the remainder of the molecule.



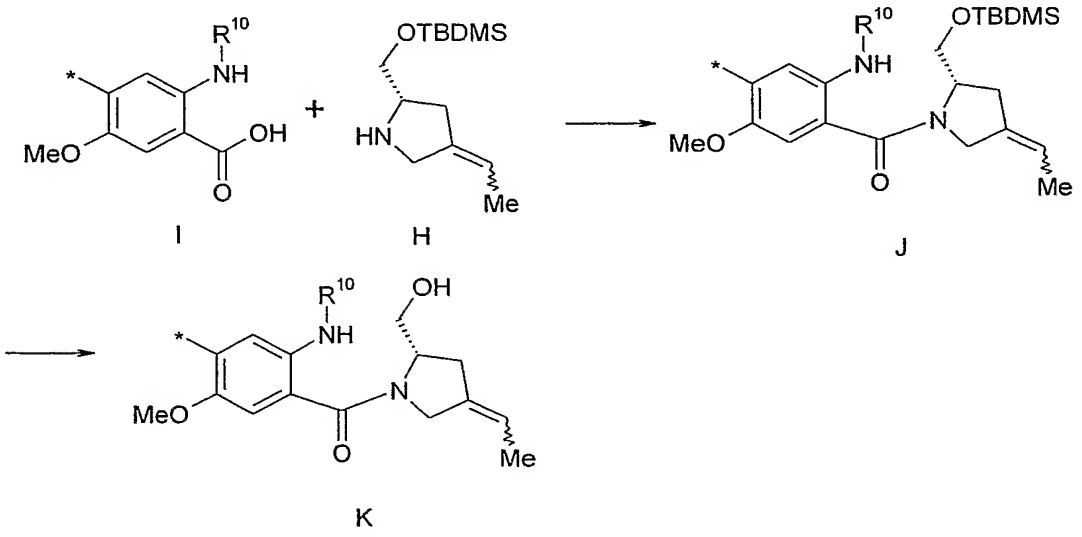
Scheme 1

Commercially available trans-4-hydroxy-L-proline **A** can be N-alloc protected (or with any other suitable nitrogen protecting group)

to give the protected compound **B** which can then be esterified using standard conditions. Hydride reduction of the ester **C** furnishes the diol **D**. Selective TBDMS protection of the diol gives a silyl ether **E**, which can then be oxidised, using, for example, Swern or TPAP oxidation, to provide the ketone **F**.

The C2-ethylidene functionality way be introduced by performing the Wittig reaction on ketone **F**. Palladium-mediated cleavage of the N-alloc protecting group yields compound **H**.

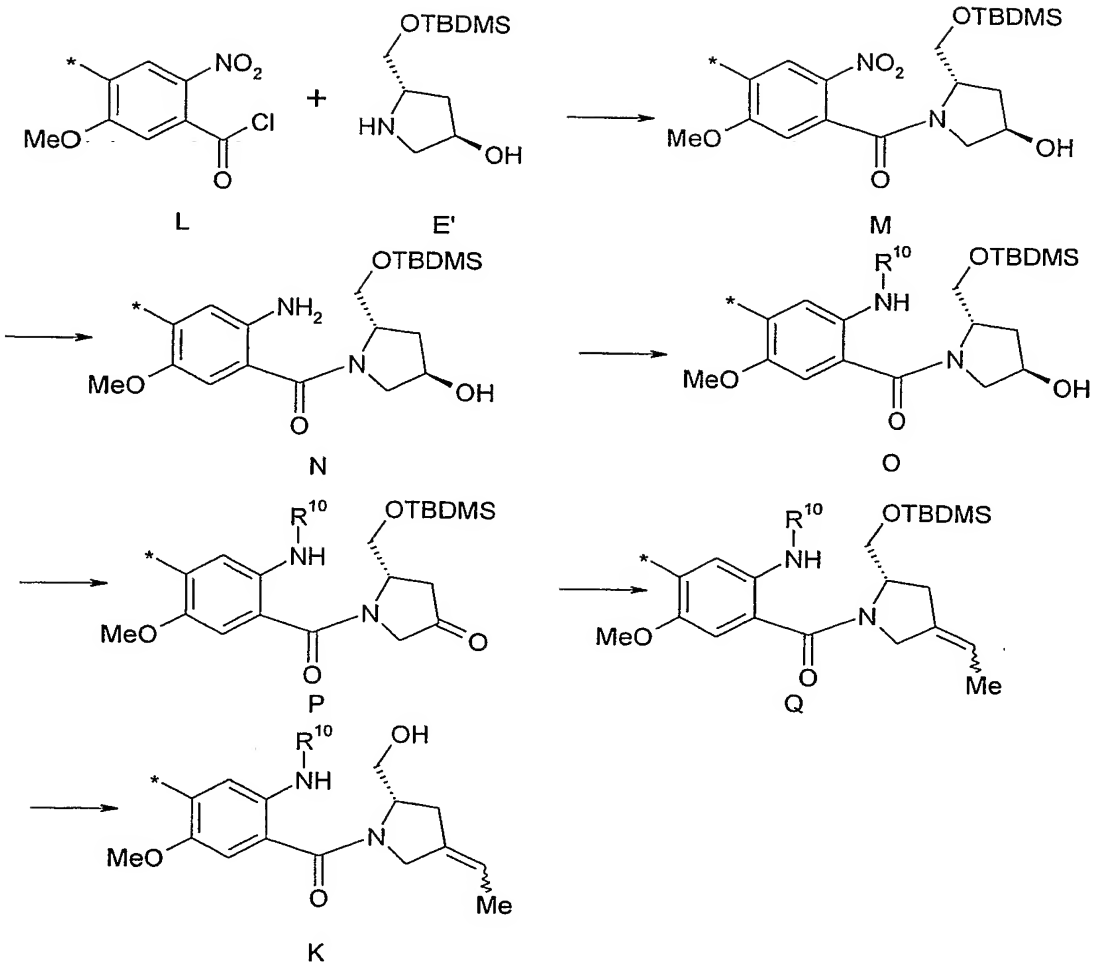
The compound H may then be joined to the A-ring dimer as follows:



Scheme 2

As shown in Scheme 2, the compound **H** is coupled (in 2 equivalents) to the N-troc protected anthranilic acid dimer **I**, where * indicates the dimer bridge (-O-$(CH_2)_5$-O-) to the corresponding PBD unit. This coupling is followed by deprotection of the alcohol to provide compound **K**.

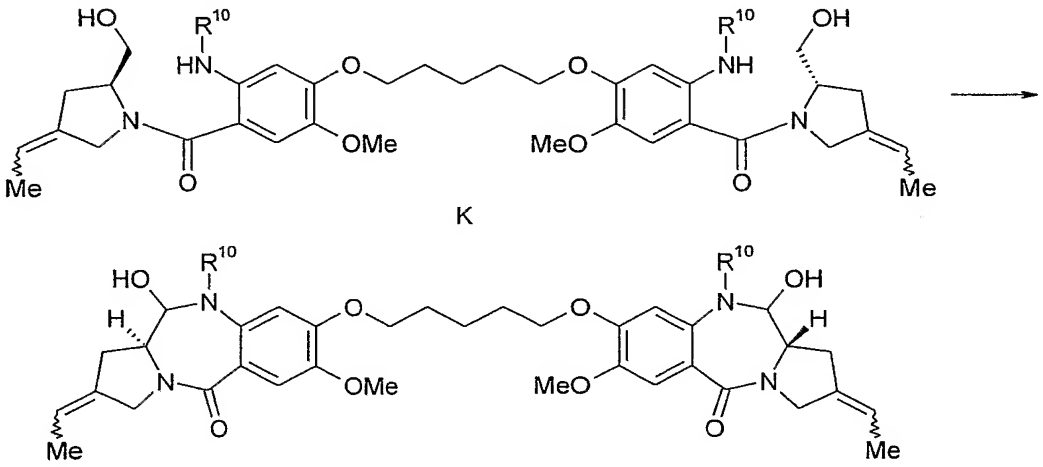
The alternative approach to compound **K** involves similar steps, but in a different order, as shown in scheme 3:



Scheme 3

The nitro dimer **M** is synthesised by coupling the amine **E'** to the dimeric acid chloride **L**. The nitro dimer is then converted to the protected aniline **O** via the aniline **N**, by reduction and then protection. The hydroxy group in the C ring can then be converted to the ketone (**P**) and subsequently ethylidene (**Q**) as described above. The compound **Q** may then be deprotected on the hydroxy to yield compound **K**.

The compound **K** may then be cylclised to yield a compound of formula **I**, wherein R^{11} is OH:



K

Exposure of the alcohol **K** (in which the Pro-N10-nitrogen is protected as carbamate) to tetrapropylammonium perruthenate (TPAP)/N-methylmorpholine N-oxide (NMO) over A4 sieves results in oxidation accompanied by spontaneous B-ring closure to afford the desired product. The TPAP/NMO oxidation procedure is found to be particularly convenient for small scale reactions while the use of DMSO-based oxidation methods, particularly Swern oxidation, proves superior for larger scale work (e.g. > 1 g). A particularly preferred oxidising agent is (diacetoxyiodo)benzene (1.1 eq) and TEMPO (0.1 eq) dissolved in CH_2Cl_2.

Alternative methods of cylisation are illustrated in WO 00/12508.

The compound of formula **I** where R^{10} is a nitrogen protecting group and R^{11} is OH may be deprotected to a compound with N10-C11 imine bonds, by removal of the nitrogen protecting groups using approprate conditions. If in the compound of formula **I**, R^{11} is O-R^{12}, then the oxygen protecting group can be introduced using the appropriate conditions.

The relative amounts of different forms of the compound of formula **I** with regard to the geometry of the C2-exo double bond may be affected by the synthesis route used, and, in particular, by the Wittig reagent used.

Further preferences

It is preferred that R^{10} and R^{11} together form a double bond between N10 and C11.

It is preferred that the compound of formula **I** comprises at least 50% in either the E-, E- or Z-, Z- forms, with more preferably at least 70%, 80%, 90% or 95% in one of these forms. The Z-, Z- form is preferred.

Examples

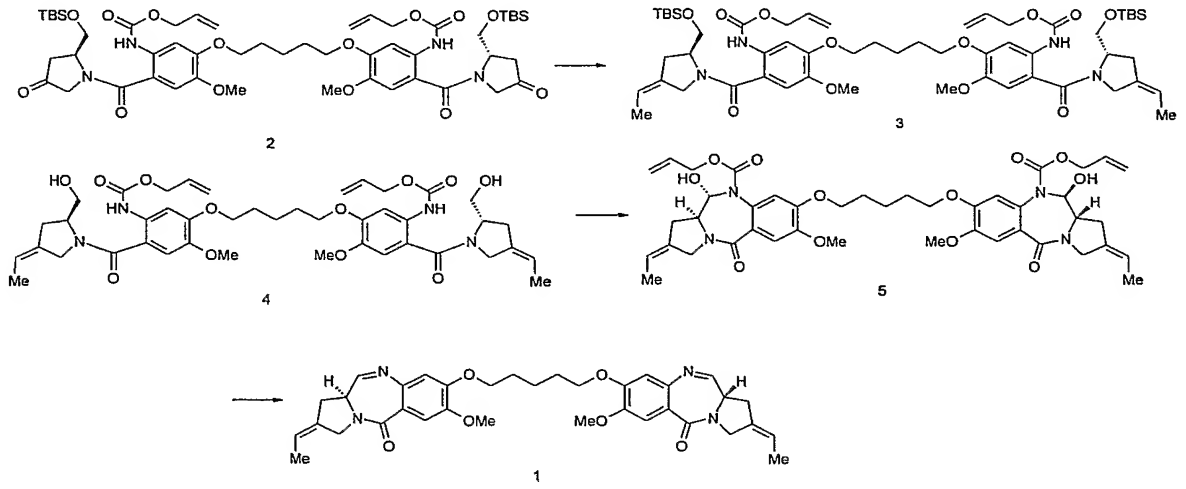
General Methods

Progress of reaction was monitored by thin-layer chromatography (TLC) using GF254 silica gel, with fluorescent indicator on glass plates. Visualization of TLC plates was achieved with UV light and I_2 vapour unless otherwise stated. Flash chromatography was performed using silica gel (14 cm column of J.T Baker 30-60 μm). The majority of reaction solvents were purified and used fresh by distillation under nitrogen from the indicated drying agent: CH_2Cl_2 and MeCN (CaH_2), tetrahydrofuran and toluene (sodium benzophenone ketyl), and MeOH (magnesium turnings and catalytic iodine). Extraction and chromatography solvents were purchased and used without further purification from J.T Baker. All organic chemicals were purchased from Aldrich Chemical Co. Drying agents and inorganic reagents were bought from BDH.

IR spectra were recorded with a Perkin-Elmer FT/IR-Paragon 1000 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a Jeol GSX 270 MHz (67.8 MHz for ^{13}C NMR spectra), Brüker ARX 250 MHz (62.9 MHz for ^{13}C NMR spectra) or Jeol JNM-LA 400 MHz (100 MHz for ^{13}C NMR spectra) FT-NMR instrument operating at 20 °C±1 °C. Chemical shifts are reported in parts per million (δ ppm) downfield from internal Me_4Si. Spin multiplicities are described as s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), t (triplet), q (quartet), quint (quintet) and m (multiplet). Mass spectra were recorded on a Jeol JMS-DX 303 GC-mass spectrometer or a VG ZAB-SE double-focusing instrument.

Electron impact (EI) mass spectra were obtained at 70 eV, chemical ionisation (CI) spectra were obtained using isobutane as reagent gas, and fast atom bombardment (FAB) spectra were recorded using 3-nitrobenzyl alcohol as a matrix with Xe reagent gas. Accurate molecular masses were determined by peak matching using perfluorokerosene (PFK) as an internal standard. Optical rotations were measured at ambient temperature using a Bellingham and Stanley ADP 220 polarimeter.

Example 1 - Synthesis of 1,1'-[(Pentane-1,5-diyl)dioxy]-bis[(11a*S*,2*Z*)-7-methoxy-2-ethylidene-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one] (1)



(a) 1,1'-[[(Pentane-1,5-diyl)dioxy]-bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylenecarbonyl]]-bis[(2S,4Z)-2-t-butyldimethylsilyloxymethyl-4-ethylidene-2,3-dihydropyrrole] (3)

A solution of potassium-*t*-butoxide in dry THF (0.5 M, 21.0 mL, 10.6 mmol) was added dropwise to a suspension of ethyltriphenylphosphonium bromide (3.94 g, 10.6 mmol) in dry THF (16 mL). The resulting yellow ylide suspension was allowed to stir at reflux for 2 hours before the addition of a solution of the bis-ketone **2** (Compound 214 from WO 00/12508)(2.09 g, 2.04 mmol) in THF (15 mL) at 10°C. The reaction mixture was allowed to stir at reflux for a further 90 minutes and then allowed to

cool to room temperature. The mixture was partitioned between EtOAc (100 mL) and water (100 mL) and the organic layer was washed with sat. sodium chloride (100 mL) and dried over $MgSO_4$. Removal of excess solvent gave a brown oil that was subjected to flash column chromatography (50:50 v/v EtOAc/40-60° petroleum ether) to afford the olefin **3** as a yellow glass. Yield = 577 mg (28%); $[\alpha]^{24}_D$ = -26° (*c* = 0.453, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 8.82 (bs, 2H), 7.81 (bs, 2H), 6.84 (s, 2H), 6.02-5.87 (m, 2H), 5.38-5.20 (m, 6H), 4.63-4.55 (m, 6H), 4.15-3.85 (m, 8H), 3.82-3.52 (m, 10H), 2.75-2.49 (m, 4H), 2.03-1.80 (m, 4H), 1.77-1.22 (m, 8H), 0.85 (s, 18H), 0.00 (s, 12H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 168.9, 153.5, 150.6, 143.9, 135.6, 132.6, 131.9, 118.0, 116.7, 115.4, 111.3, 105.4, 68.6, 65.7, 63.7, 56.6, 54.6, 33.4, 28.8, 25.8, 22.6, 18.1, 14.6, -5.58; MS (FAB) *m/z* (relative intensity) 1072 ($[M + Na + H]^{+\cdot}$, 65), 1049 ($[M + H]^{+\cdot}$, 28), 992 (13), 809 (39), 509 (33), 469 (49), 318 (26), 268 (100); IR (Neat) 3319 (br), 2952, 2930, 2858, 1732, 1600, 1524, 1470, 1407, 1360, 1331, 1258, 1202, 1115, 1052, 1027, 938, 837, 812, 666 cm^{-1}; HRMS $[M + Na]^{+\cdot}$ calcd for $C_{55}H_{84}N_4O_{12}Si_2Na$ *m/z* 1071.5522, found (FAB) *m/z* 1071.5468.

*(b) 1,1′-[[(Pentane-1,5-diyl)dioxy]-bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylenecarbonyl]]-bis[(2S,4Z)-2-hydroxymethyl-4-ethylidene-2,3-dihydropyrrole] (**4**)*

A solution of TBAF (3.00 mL of a 1.0 M solution in THF, 3.00 mmol) was added to the bis-silyl ether **3** (1.23 g, 1.21 mmol) in THF (30 mL) at 0°C (ice/acetone). The reaction mixture was allowed to warm to room temperature and to stir overnight, the following day, TLC (50:50 v/v EtOAc/40-60° petroleum ether) revealed the complete disappearance of starting material. Saturated NH_4Cl (150 mL) was added and the reaction mixture extracted with EtOAc (3 x 60 mL), washed with sat. sodium chloride (150 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give a yellow oil. Purification by flash chromatography (97:3 v/v $CHCl_3$/MeOH) provided the pure alcohol **4** as a white foam. Yield = 879 mg (91%); $[\alpha]^{23}_D$ = -2° (*c* = 0.29, $CHCl_3$); ^1H NMR

(250 MHz, $CDCl_3$) δ 8.64 (bs, 2H), 7.58 (bs, 2H), 6.82 (bs, 2H), 6.04-5.88 (m, 2H), 5.41-5.21 (m, 6H), 4.71-4.56 (m, 6H), 4.12-3.60 (m, 20H), 2.72 (dd, 2H, J = 8.2, 15.1 Hz), 2.38 (d, 2H, J = 15.3 Hz), 2.00-1.89 (m, 4H), 1.75-1.50 (m, 8H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 170.5, 153.7, 150.4, 144.5, 134.2, 132.6, 130.9, 118.0 (x 2), 116.2, 110.9, 106.0, 68.5, 65.7, 65.3, 59.4, 56.6, 51.0, 34.1, 28.6, 22.7, 14.6; MS (FAB) *m/z* (relative intensity) 843 ($[M + Na]^{+\cdot}$, 100), 821 ($[M + H]^{+\cdot}$, 17), 694 (32), 509 (43), 469 (40), 421 (25), 336 (50), 307 (34); IR ($CHCl_3$) 3355 (br), 3016, 2941, 2875, 1723, 1600, 1525, 1465, 1434, 1409, 1330, 1266, 1216, 1179, 1118, 1072, 1051, 1028, 995, 933, 872, 667 cm^{-1}; HRMS $[M + Na]^{+\cdot}$ calcd for $C_{43}H_{56}N_4O_{12}Na$ *m/z* 843.3792, found (FAB) *m/z* 843.3823.

*(c) 1,1'-[(Pentane-1,5-diyl)dioxy]-bis[(11S,11aS,2Z)-10-(allyloxycarbonyl)-11-hydroxy-7-methoxy-2-ethylidene-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one] **(5)***

A solution of dimethyl sulphoxide (0.45 g, 0.41 mL, 5.80 mmol) in dry CH_2Cl_2 (8 mL) was added dropwise, over a 15 minute period, to a stirred solution of oxalyl chloride (1.46 mL of a 2M solution in CH_2Cl_2, 2.92 mmol) at -45°C under a nitrogen atmosphere. The reaction mixture was allowed to stir for 35 minutes at -45°C followed by addition of the diol **4** (0.85 g, 1.04 mmol) in CH_2Cl_2 (8 mL) at the same temperature over 15 minutes. After a further 45 minutes a solution of triethylamine (0.83g, 1.14 mL, 8.20 mmol) in CH_2Cl_2 (8 mL) was added over a period of 15 minutes. The reaction mixture was allowed to stir at -45°C for 30 minutes before being allowed to warm to room temperature over 45 minutes. The reaction mixture was diluted with CH_2Cl_2 and was washed with 1M HCl (3 x 50 mL), brine (50 mL) and dried over $MgSO_4$. Removal of excess solvent yielded the crude product, which was purified by flash column chromatography (99:1 v/v $CHCl_3$/MeOH) to afford the product as a white glass **5**. Yield = 0.495 g (58%); $[\alpha]^{22}_D$ = +168° (*c* = 0.28, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 2H), 6.66 (s, 2H), 5.90-5.70 (m, 2H), 5.59-5.40 (m, 4H), 5.16 (bs, 2H), 5.11 (bs, 2H), 4.66 (dd, 2H, J = 5.57, 13.59 Hz), 4.44 (d,

2H, J = 13.2 Hz), 4.29-4.07 (m, 6H), 4.01 (t, 4H, J = 6.5 Hz), 3.90 (s, 6H), 3.63-3.56 (m, 2H), 2.93-2.77 (m, 2H), 2.66 (d, 2H, J = 16.4 Hz), 1.97-1.86 (m, 4H), 1.68-1.61 (m, 8H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 166.8, 155.9, 150.2, 148.8, 133.0, 131.8, 128.4, 125.5, 119.6, 118.2, 113.9, 110.6, 85.9, 69.0, 66.8, 59.3, 56.1, 47.5, 34.8, 28.5, 22.4, 14.8; MS (FAB) *m/z* (relative intensity) 839 ([M + Na]$^{+\cdot}$, 100), 799 (10), 781 (14), 465 (14), 443 (16), 413 (37), 388 (19), 336 (25), 271 (25); IR ($CHCl_3$) 3225 (br), 3011, 2938, 2860, 1704, 1605, 1515, 1469, 1436, 1410, 1307, 1284, 1215, 1129, 1077, 1018, 994, 959, 916, 872, 666, 637 cm^{-1}; HRMS [M + Na]$^{+\cdot}$ calcd for $C_{43}H_{52}N_4O_{12}Na$ *m/z* 839.3479, found (FAB) *m/z* 839.3497.

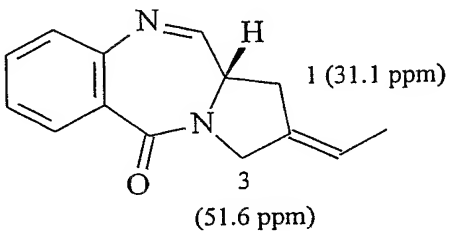
*(d) 1,1'-[(Pentane-1,5-diyl)dioxy]-bis[(11aS,2Z)-7-methoxy-2-ethylidene-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one] (**1**)*

A catalytic amount of tetrakis(triphenylphosphine)palladium (14.4 mg, 12.5 μmol) was added to a stirred solution of the *bis*-alloc-carbinolamine **5** (200 mg, 0.25 mmol), triphenylphosphine (6.30 mg, 24.1 μmol) and pyrrolidine (33 mg, 40.1 μL 0.48 mmol) in CH_2Cl_2 (13 mL) under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and the progress of reaction monitored by TLC (95:5 v/v $CHCl_3$/MeOH). After two and a half hours TLC revealed the reaction was complete to give a spot which fluoresced brightly under UV light. The solvent was evaporated under reduced pressure and the resulting residue subjected to flash chromatography (98:2 v/v $CHCl_3$/MeOH) to give the bis-imine target molecule **1** as a pale orange glass which was repeatedly evaporated *in vacuo* with $CHCl_3$ to provide the imine form. Yield = 160 mg (Quant); $[\alpha]^{21}_D$ = +937° (c = 0.641, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.67 (d, 2H, J = 4.5 Hz), 7.50 (s, 2H) 6.80 (s, 2H), 5.63-5.55 (m, 2H), 4.38-3.96 (m, 8H), 3.94 (s, 6H), 3.86-3.80 (m, 2H), 3.20-3.03 (m, 2H), 2.90 (d, 2H, J = 15.8 Hz), 2.00-1.91 (m, 4H), 1.76-1.68 (m, 8H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.9, 163.0, 150.8, 147.8, 140.6, 132.9, 119.8, 119.3, 111.4, 110.3, 68.7, 56.1, 53.4, 48.3, 35.3, 28.6, 22.5, 14.9; MS (FAB)

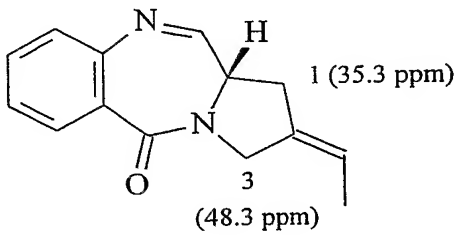
m/z (relative intensity) 613 ([*M* + H]$^{+\cdot}$, 52), 443 (26), 421 (22), 329 (100), 307 (71), 242 (35), 220 (38); IR ($CHCl_3$) 3220 (br), 2940, 2859, 1697, 1602, 1560, 1508, 1458, 1432, 1382, 1341, 1263, 1217, 1132, 1098, 1065, 1007, 875, 666 cm^{-1}; HRMS [*M* + H]$^{+\cdot}$ calcd for $C_{35}H_{41}N_4O_6$ *m/z* 613.3026, found (FAB) *m/z* 613.3047.

Determination of relative amounts of geometric isomers

The chemical shift differences observed in the ^{13}C NMR spectra for the C1 and C3 resonances of the E/Z ethylidene group of the PBD allows the determination of approximate geometric isomer ratios in compound **1**. These observations are based on work published on the total synthesis of the PBD natural products tomaymycin and prothracarcin which contain C2-ethylidene moieties (Mori, M., *et al.*, *Tetrahedron*, **42**, 3793 (1986)).



E-prothracarcin



Z-prothracarcin

Table 1 shows a comparison of ^{13}C NMR signals for C1 and C3 of E- and Z-prothracarcin and *E/Z* forms of the C2-*exo* double bond of Compound **1**. The relative signal intensities measured for Compound **1** are quoted in parentheses.

	Chemical Shift (ppm)			
	E- prothracarcin	*Z*- prothracarcin	*E*-**1**	*Z*-**1**
C1	31.1	35.3	31.1 (0.21)	35.3 (2.60)
C3	51.6	48.3	51.6 (0.20)	48.3 (2.84)

Table 1

From these data the approximate amount of C2 *exo* double bond in the Z- form is 93.6% and in the E- form is 6.4% which give the relative amounts of geometric isomers of compound **1** as below:

Geometric isomers at C2/C2'	Amount (%)
E-, E-	0.4
E-, Z-	12
Z-, Z-	87.6

In addition, NOESY (through space correlations) spectra on compound **1** supports the structural assignment.

Example 2 - Biological Evaluation

K562 Assay

K562 human chronic myeloid leukaemia cells were maintained in RPM1 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine at 37°C in a humidified atmosphere containing 5% CO_2 and were incubated with a specified dose of the test compound for 1 hour at 37°C in the dark. The incubation was terminated by centrifugation (5 minutes, 300 *g*) and the cells were washed once with drug-free medium. Following the appropriate drug treatment, the cells were transferred to 96-well microtiter plates (10^4 cells per well, 8 wells per sample). Plates were then kept in the dark at 37°C in a humidified atmosphere containing 5% CO_2.

The assay is based on the ability of viable cells to reduce a yellow soluble tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Aldrich-Sigma), to an insoluble purple formazan precipitate. Following incubation of the plates for 4 days (to allow control cells to increase in number by approximately 10 fold), 20 µL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well and the plates further incubated for 5 hours. The plates were then centrifuged for 5 minutes at 300 g and the bulk of the medium pipetted from the cell pellet leaving 10-20 µL per well. DMSO (200 µL) was added to each well and the samples agitated to ensure complete mixing. The optical density was then read at a wavelength of 550 nm on a Titertek Multiscan ELISA plate reader, and a dose-response curve was constructed. For each curve, an IC_{50} value was read as the dose required to reduce the final optical density to 50% of the control value.

The IC_{50} value measured for compound **1** was >0.05nM, which compares to a value for the compound of Example 6 in WO 93/18045 quoted as 10 nM.

DNA Cross-linking Assay

The extent of DNA cross-linking induced by the test compound was determined using the electrophoretic assay method of Hartley and co-workers (Hartley, J. A., *et al.*, *Analytical Biochemistry,* **193**, 131-134 (1991)). Closed-circular puc18 DNA was linearized with HindIII, then dephosphorylated and finally 5'-singly end-labelled using [γ^{32}P]-ATP and polynucleotide kinase. Reactions containing 30-40 ng of DNA were carried out in aqueous TEOA (25 mM triethanolamine, 1 mM EDTA, pH 7.2) buffer at 37°C in a final volume of 50 µL. Reactions were terminated by addition of an equal volume of stop solution (0.6 M NaOAc, 20 mM EDTA, 100 µg/mL tRNA) followed by precipitation with EtOH. Following centrifugation, the supernatant was discarded and the pellet dried by lyophilization. Samples were re-suspended in 10 µL of strand separation buffer (30% DMSO, 1 mM EDTA, 0.04% bromophenol blue

and 0.04% xylenecyanol) and denatured by heating to 90°C for 2.5 minutes, followed by immersion in an ice/water bath. Control, non-denatured, samples were re-suspended in 10 µL of non-denaturing buffer solution (0.6% sucrose, 0.04% bromophenol blue in aqueous TAE buffer [40 mM Tris, 20 mM acetic acid, 2 mM EDTA, pH 8.1]) and loaded directly onto the gel for comparison. Electrophoresis was carried out for 14-16 h at 40 V using a 0.8% submerged agarose gel (20 × 25 × 0.5 cm) in TAE buffer. Gels were dried under vacuum for 2 hours at 80°C onto one layer each of Whatman 3MM and DE8I filter papers using a BioRad 583 gel dryer. Autoradiographs were obtained after exposure of Hyperfilm-MP film (Amersham plc, U.K.) to the dried gel for either 4 h with a screen, or overnight without a screen (to obtain a sharper image). Film bands were quantitated using a BioRad GS-670 imaging laser densitometer. Percentage cross-linking was calculated by measuring the total DNA in each lane (summed density for the double-stranded [DS] and single-stranded [SS] bands) relative to the amount of cross-linked DNA (density of DS band alone). A dose-response curve was derived by plotting drug concentration against the determined percentage level of cross-linked DNA, and the result XL_{50} determined as the amount required to cross-link 50%.

The XL_{50} determined for compound **1** was 2.7 ± 1.6 nM.